

WHAT IS CLAIMED IS:

1. A method for reducing the effect of inhibitory/instability sequences within the coding region of a mRNA, said method comprising the steps of:

- (a) providing a gene which encodes said mRNA;
- (b) identifying the inhibitory/instability sequences within said gene which encode said inhibitory/instability sequences within the coding region of said mRNA;
- (c) mutating said inhibitory/instability sequences within said gene by making multiple point mutations;
- (d) transfecting said mutated gene into a cell;
- (e) culturing said cell in a manner to cause expression of said mutated gene;
- (f) detecting the level of expression of said gene to determine whether the effect of said inhibitory/instability sequences within the coding region of the mRNA has been reduced.

2. The method of Claim 1 further comprising the step of fusing said mutated gene to a reporter gene prior to said transfecting step and said detecting step is performed by detecting the level of expression of said reporter gene.

3. The method of Claim 1 wherein step (b) further comprises the steps of

- (a) fusing said gene or fragments of said gene to a reporter gene to create a fused gene;
- (b) transfecting said fused gene into a

- cell;
- (c) culturing said cell in a manner to cause expression of said fused gene;
- (d) detecting the level of expression of said fused gene to determine whether the expression of said fused gene is reduced relative to the expression of said reporter gene.

4. The method of Claim 3 wherein step (a) comprises fusing said gene or fragments of said gene 3' to the stop codon of said reporter gene.

5. The method of Claim 3 wherein step (a) comprises fusing said gene or fragments of said gene in frame with the 3' end of the coding region of said reporter gene.

6. The method of Claim 1 or 2 wherein said mutating step changes the codons such that the amino acid sequence encoded by the mRNA is unchanged.

7. The method of Claim 6 wherein said inhibitory/instability sequences are AT-rich and wherein said mutating step comprises substituting either G or C for either A or T and wherein the final nucleotide composition of said mutated inhibitory sequence is about 50% A and T and about 50% G and C.

8. The method of Claim 6 wherein at least 75% of the point mutations replace conserved nucleotides with non-conserved nucleotides.

9. The method of Claim 6 wherein said mutating step comprises substituting less preferred codons with more preferred codons.

10. The method of Claim 1 or 2 wherein said mRNA encodes the GAG protein of a Rev-dependent complex retrovirus.

5 11. The method of Claim 10 wherein the Rev-dependent complex retrovirus is human immunodeficiency virus-1.

10 12. A method of increasing the production of a polypeptide, wherein said polypeptide is encoded by a mRNA that contains one or more inhibitory/instability sequences, said method comprising the steps of:

- 15 (a) providing a gene which encodes said mRNA;
- (b) identifying the inhibitory/instability sequences within said gene which encode said inhibitory/instability sequences within the coding region of said mRNA;
- 20 (c) mutating said inhibitory/instability sequences within said gene by making multiple point mutations;
- (d) transfecting said mutated gene into a cell;
- 25 (e) culturing said cell in a manner to cause expression of said mutated gene;
- (f) detecting the level of expression of said gene to determine that the effect of said inhibitory/instability sequences within the coding region of the mRNA has been reduced;
- 30 (g) providing a host cell transfected with an expression vector containing said mutated gene;
- 35 (h) culturing said host cell to cause expression of said polypeptide; and

(i) recovering said polypeptide.

13. A method of producing polypeptides, -whose native production is impeded by the presence of an inhibitory/instability sequence, comprising the steps of:

- 5 (a) providing a host cell transfected with an expression vector containing a gene encoding said polypeptide, said gene having been mutated to decrease the effect of the inhibitory/instability sequence;
- 10 (b) culturing said host cell to cause expression of said polypeptide; and
- (c) recovering said polypeptide.

15 14. The method of Claim 13 wherein said host cell is prokaryotic.

20 15. The method of Claim 13 wherein said host cell is eukaryotic.

16. The method of Claims 13, 14 or 15 wherein said gene is a cDNA.

25 17. The method of Claims 13, 14 or 15 wherein said gene is genomic.

30 18. An artificial nucleic acid construct comprising a gene wherein the expression of the native gene is impeded by the presence of inhibitory/instability sequences in the mRNA encoded by said native gene, said gene having being mutated to decrease the effect of the inhibitory/instability sequence.

35 19. The construct of Claim 18 wherein the amino acid sequence encoded by said mutated gene is the same as

the amino acid sequence encoded by the native gene.

20. The construct of Claim 19 wherein said native gene is HIV-1 gag.

21. The construct of Claim 20 wherein said HIV-1 gag gene has been mutated by the introduction of multiple point mutations between nucleotides 402 and 452, 536 and 583, 585 and 634, and 654 and 703.

22. The construct of claim 19 wherein said native gene is HIV-1 env.

23. An assay kit for identifying inhibitory/instability sequences in a mRNA, comprising:

- (a) the nucleic acid construct of Claim 20 or 21; and
- (b) a detection system for detecting the level of expression of said gene in said nucleic acid construct.

24. The kit of Claim 23 wherein said detection system is an ELISA.

25. An artificial nucleic acid construct comprising a gene mutated by the method of Claim 1 or 2.

26. A vector comprising the nucleic acid construct of Claim 25.

27. A transformed host cell comprising the artificial nucleic acid construct of Claim 25.

28. A vector comprising the nucleic acid construct of Claim 18 or 19.

29. A transformed host cell comprising the artificial nucleic acid construct of Claim 18 or 19.

5 30. A transformed host cell of Claim 29 wherein said cell is selected from the group consisting of eukaryotes and prokaryotes. A

31. The host cell of Claim 30 wherein said cell is a human cell.

10 32. The host cell of Claim 30 wherein said cell is a Chinese Hamster Ovary cell.

15 33. The host cell of Claim 30 wherein said cell is E. coli.

20 34. The construct of Claim 20 wherein said HIV-1 gag gene has been mutated by the introduction of multiple point mutations between nucleotides 402 and 452, 536 and 583, 585 and 634, 654 and 703, 871 and 915, 1105 and 1139, 1140 and 1175 and 1321 and 1364.

35. The construct of Claim 34 wherein said HIV-1 gag gene is p37M1-10D. A

25 36. The construct of Claim 20 wherein said HIV-1 gag gene has been mutated by the introduction of multiple point mutations between nucleotides 402 and 452, 536 and 583, 585 and 634, 654 and 703, 871 and 915, 1105 and 1139, 1140 and 1175, 1321 and 1364, 1416 and 1466,  
30 1470 and 1520, 1527 and 1574, and 1823 and 1879.

37. The construct of Claim 36 wherein said HIV-1 gag gene is p55M1-13P0.

35 38. A vaccine composition for inducing immunity

in a mammal against HIV infection comprising a pharmaceutically acceptable medium and further comprising a therapeutically effective amount of a nucleic acid construct capable of producing HIV gag protein in the absence of any HIV regulatory protein in a cell in vivo.

5

39. A vaccine composition according to claim 38 wherein said mammal is a human.

10

40. A vaccine composition according to claim 38 wherein said regulatory protein is HIV-1 Rev.

15

41. A vaccine composition according to claim 38 wherein said construct is selected from the group consisting of the construct of claim 20, 21, 34, 35, 36, and 37.

20

42. A method for inducing immunity against HIV infection in a mammal which comprises administering to a mammal a therapeutically effective amount of a vaccine composition comprising a nucleic acid construct capable of producing HIV gag protein in the absence of any HIV regulatory protein in a cell in vivo.

25

43. A method according to claim 42 wherein said mammal is a human.

44. A method according to claim 42 wherein said regulatory protein is HIV-1 Rev.

30

45. A method according to claim 42 wherein said construct is selected from the group consisting of the construct of claim 20, 21, 34, 35, 36, and 37.

35

Add  
A2